

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO.
42-R-0009

CUSTOMER NO.
1578

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA, include Zip Code)

FORT DODGE LABORATORIES
800 5TH ST N W
FORT DODGE IA 50501

(B)(6) (B)(7)(c)

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS/sites

See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain- relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs	164	934	77	131	1142
5. Cats		1244	55	243	1542
6. Guinea Pigs		3527	11	0	3538
7. Hamsters		15200	872	7261	23333
8. Rabbits		158	1965	0	2123
9. Non-Human Primates		0	0	0	0
10. Sheep	3	0	0	0	0
11. Pigs		53	0	0	53
12. Other Farm Animals Cattle		58	0	0	58
Horses		11	0	0	11
13. Other Animals Goats		5	0	0	5
Gerbils		90	230	0	320

ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all the exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

NOV 27 2007 ✓

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

ATE SIGNED

Nov 27

QUARTERS

(B)(6) (B)(7)(c)

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 24
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and inoculated with a virulent strain of B4
The dogs were allowed to develop clinical signs of the infection so that observations and sampling could be made and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted for the qualification of a new master reference vaccine. Product- and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease.
This information would be used to qualify this reference vaccine. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the leptospira organism. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

A master or working reference is necessary for *in vitro* potency testing for product release of B4 containing products. VS Memorandum 800.90 III.A. "A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity". 9CFR 113.8 (d)(2) requires the use of an unexpired reference for *In vitro* tests for serial release.

VS Memorandum 800.202 3.6.1 "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement".

VS Memorandum 800.202 1.3 "Efficacy is the direct effect of a medical intervention on an individual subject".

VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo-controlled, randomized, and double blinded. Vaccine trials should preferably aim to compare product- and placebo-treated subjects by their response to challenge with virulent pathogen.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species: Canine**
2. **Number of animals achieving Cat. E in this study: 4**
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were inoculated B4 under general anesthesia, with the B4 The dogs were observed for clinical signs of B4
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted to develop a challenge model. A challenge model is the method used to administer the pathogen to animals so they will exhibit the clinical signs of infectious disease. This information would be used to qualify a reference vaccine. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by B4. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with analgesics, non-steroidal anti-inflammatories, B4 would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

A master or working reference is necessary for *in vitro* potency testing for product release of B4 containing products. VS Memorandum 800.90 III.A. "A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity". 9CFR 113.8 (d)(2) requires the use of an unexpired reference for *In vitro* tests for serial release.

VS Memorandum 800.202 3.6.1 "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement".

VS Memorandum 800.202 1.3 "Efficacy is the direct effect of a medical intervention on an individual subject".

VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo-controlled, randomized, and double blinded. Vaccine trials should preferably aim to compare product- and placebo-treated subjects by their response to challenge with virulent pathogen.

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 20
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and B4 virus was administered B4
The dogs were allowed to develop the B4 clinical signs of the infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease.
This information would be used to establish the possible efficacy of several vaccine candidates. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antitussives would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 10
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and B4 was administered B4
The dogs were allowed to develop the B4 clinical signs of the infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted to develop a challenge model. A challenge model is the method used to administer the pathogen to animals so they will exhibit the clinical signs of infectious disease. This information would be used to establish vaccine efficacy and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by B4. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease. However treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, antitussives and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 40
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and B4 virus was administered B4
The dogs were allowed to develop the B4 clinical signs of the infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antitussives would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 15
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and B4 virus was administered B4 .
The dogs were allowed to develop the B4 clinical signs of the infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted to develop a challenge model. A challenge model is the method used to administer the pathogen to animals so they will exhibit the clinical signs of infectious disease. This information would be used to establish vaccine efficacy and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease. However treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, antitussives and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 4
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized B4 ; was administered B4 The dogs were observed for the first signs of B4 At the first signs of infection the dogs could be euthanized with a barbiturate overdose. However these 4 animals did not exhibit any signs of B4 infection on one day of observation and were dead the next day of observation before they could be euthanized.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Vaccinated and non-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish duration of immunity and support label claims. The pain and/or distress from this infectious disease could have been relieved with euthanasia using a barbiturate overdose if the signs of infection were observed before death.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: 4.5 Species-Establish efficacy in each species for which the product is recommended. 4.6 Age and susceptibility-Conduct target species immunogenicity studies in fully susceptible animals of the youngest age for which the product is recommended. 4.8 Duration of immunity- Conduct duration of immunity studies to support vaccination recommendations for all new product fractions presented for licensure.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 6
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and B4 was administered B4. The dogs were observed for the first signs of B4. At the first signs of infection the dogs could be euthanized with a barbiturate overdose. However these 6 animals did not exhibit any signs of B4 infection on one day of observation and were dead the next day of observation before they could be euthanized.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Vaccinated and non-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and support label claims. The pain and/or distress from this infectious disease could have been relieved with euthanasia using a barbiturate overdose if the signs of infection were observed before death.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: 4.5 Species-Establish efficacy in each species for which the product is recommended. 4.6 Age and susceptibility-Conduct target species immunogenicity studies in fully susceptible animals of the youngest age for which the product is recommended.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Canine
2. **Number of animals achieving Cat. E in this study:** 8
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Dogs were anesthetized and B4 was administered B4 The dogs were observed for the first signs B4 infection. At the first signs of infection the dogs could be euthanized with a barbiturate overdose. However these eight animals did not exhibit any signs of rabies infection on one day of observation and were dead the next day of observation before they could be euthanized.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted to develop a challenge model. A challenge model is the method used to administer the pathogen to animals so they will exhibit the clinical signs of infectious disease. This information would be used to establish vaccine efficacy, duration of immunity and support label claims. The pain and/or distress from this infectious disease could have been relieved with euthanasia using a barbiturate overdose if the signs of infection were observed before death.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: 4.5 Species-Establish efficacy in each species for which the product is recommended. 4.6 Age and susceptibility-Conduct target species immunogenicity studies in fully susceptible animals of the youngest age for which the product is recommended. 4.8 Duration of immunity- Conduct duration of immunity studies to support vaccination recommendations for all new product fractions presented for licensure.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 10
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 The cats were allowed to develop the clinical signs of the infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish duration of immunity and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, antitussives and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum, specifically 4.7 duration of immunity.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 5
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and B4 was administered B4 The cats were observed for the first signs of B4 . At the first signs of infection the cats could be euthanized with a barbiturate overdose. However these 5 animals did not exhibit any signs of rabies infection on one day B4 and were dead the next day of observation before they could be euthanized.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Vaccinated and non-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish duration of immunity and support label claims. The pain and/or distress from this infectious disease could have been relieved with euthanasia using a barbiturate overdose if the signs of infection were observed before death.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: 4.5 Species-Establish efficacy in each species for which the product is recommended. 4.6 Age and susceptibility-Conduct target species immunogenicity studies in fully susceptible animals of the youngest age for which the product is recommended. 4.8 Duration of immunity- Conduct duration of immunity studies to support vaccination recommendations for all new product fractions presented for licensure.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 4
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized ; B4 virus was administered B4 The cats were observed for the first signs B4 infection. At the first signs of infection the cats could be euthanized with a barbiturate overdose. However these 4 animals did not exhibit any signs of B4 on one day of observation and were dead the next day of observation before they could be euthanized.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Vaccinated and non-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish duration of immunity and support label claims. The pain and/or distress from this infectious disease could have been relieved with euthanasia using a barbiturate overdose if the signs of infection were observed before death.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: 4.5 Species-Establish efficacy in each species for which the product is recommended. 4.6 Age and susceptibility-Conduct target species immunogenicity studies in fully susceptible animals of the youngest age for which the product is recommended. 4.8 Duration of immunity- Conduct duration of immunity studies to support vaccination recommendations for all new product fractions presented for licensure.

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 20
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with B4 The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with the organism to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and determine the onset of immunity. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 20
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with B4
The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to determine the duration of immunity. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 17
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with B4
The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to determine efficacy and onset of immunity. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 10
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with various strains of B4 virus. The cats were allowed to develop the clinical signs of the infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted to determine the clinical signs of infection caused by different B4 viruses. This information would be used to select a possible vaccine candidate and develop a challenge model to establish vaccine efficacy and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 19
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with B4 virus. The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and determine the onset of immunity. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 11
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with B4 virus. The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and determine the onset of immunity. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 16
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 with B4 virus.
The cats were allowed to develop the clinical signs of the respiratory infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to determine efficacy. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 10
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 ith B4 The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
B4

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 20
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated B4 . The cats were allowed to develop the clinical signs of the B4 infection. The clinical signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product-and placebo-vaccinated animals were challenged with the organism to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 19
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were anesthetized and inoculated. B4 The cats
were allowed to develop the clinical signs of the B4 infection. The clinical
signs were observed and recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Product- and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and determine the onset of immunity. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 27
3. **Explanation of the procedure producing pain and/or distress:**
Cats were inoculated, under general anesthesia, with a virulent strain of [REDACTED] B4
B4 known to cause feline B4 disease in cats. The cats were observed for clinical
signs of feline B4 disease.
4. **State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Scientific justification why pain and/or distress could not be relieved.**
This animal study was conducted for the qualification of a new master reference vaccine. Product- and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy of the reference vaccine. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

A master or working reference is necessary for *in vitro* potency testing for product release of FCV containing products. VS Memorandum 800.90 III.A. "A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity".
9CFR 113.8 (d)(2) requires the use of an unexpired reference for *In vitro* tests for serial release.

VS Memorandum 800.202 3.6.1 "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement".

VS Memorandum 800.202 1.3 "Efficacy is the direct effect of a medical intervention on an individual subject".

VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo-controlled, randomized, and double blinded. Vaccine trials should preferably aim to compare product- and placebo-treated subjects by their response to challenge with virulent pathogen.

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species: Feline**
2. **Number of animals achieving Cat. E in this study: 17**
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Cats were inoculated B4 under general anesthesia, with the B4 The cats were observed for clinical signs of infection of B4.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted for the qualification of a new master reference vaccine. Product-and placebo-vaccinated animals were challenged with protozoa to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy of the reference vaccine. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by B4. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with analgesics, non-steroidal anti-inflammatories, corticosteroids B4 would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

A master or working reference is necessary for *in vitro* potency testing for product release of B4 containing products. VS Memorandum 800.90 III.A. "A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity". 9CFR 113.8 (d)(2) requires the use of an unexpired reference for *In vitro* tests for serial release.

VS Memorandum 800.202 3.6.1 "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement".

VS Memorandum 800.202 1.3 "Efficacy is the direct effect of a medical intervention on an individual subject".

VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo-controlled, randomized, and double blinded. Vaccine trials should preferably aim to compare product- and placebo-treated subjects by their response to challenge with virulent pathogen.

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Feline
2. **Number of animals achieving Cat. E in this study:** 18
3. **Explanation of the procedure producing pain and/or distress:**
Cats were inoculated, under general anesthesia, with a virulent strain of B4
B4 known to cause feline r B4 in cats. The cats were observed for
clinical signs of feline B4
4. **State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Scientific justification why pain and/or distress could not be relieved.**
This animal study was conducted for the qualification of a new master reference vaccine. Product- and placebo-vaccinated animals were challenged with virus to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the virus. The attending veterinarian was consulted for possible therapeutic treatment for the pain and distress of the disease but treatment with antipyretics, analgesics, non-steroidal anti-inflammatories, corticosteroids, and antibiotics would alter the expression of the disease.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

A master or working reference is necessary for *in vitro* potency testing for product release of B4 containing products. VS Memorandum 800.90 III.A. "A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity". 9CFR 113.8 (d)(2) requires the use of an unexpired reference for *In vitro* tests for serial release.

VS Memorandum 800.202 3.6.1 "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement".

VS Memorandum 800.202 1.3 "Efficacy is the direct effect of a medical intervention on an individual subject".

VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo-controlled, randomized, and double blinded. Vaccine trials should preferably aim to compare product- and placebo-treated subjects by their response to challenge with virulent pathogen.

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Hamsters
2. **Number of animals achieving Cat. E in this study:** 43
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Five hamsters per serial are vaccinated : B4 After 15-20 days, the hamsters are challenged B4 with an appropriate dilution of virulent B4 preparation. Five non-vaccinated hamsters are given the same challenge dose and used as controls. Four groups of five non-vaccinated hamsters are given a dilution of the challenge material and used as the challenge titration determination. The hamsters are observed for at least 14 days after the death of four control hamsters and deaths are recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
The test is required by regulation as a proof of B4 vaccine potency to be conducted on each serial of vaccine produced. Death of hamsters in this test is used to indicate lack of protection from B4 . Because the vaccine is given at a fractional dose, the test amounts to a protective endpoint determination for the vaccine being tested. B4 in hamsters almost always results in acute onset and rapid death. The rapid progression of the disease in the hamster gives little opportunity for intervention. Furthermore, pathology and signs, length and severity of clinical disease would be impacted by use of non-steroidal anti-inflammatories, antibiotics, corticosteroids, and analgesics. For this reason Fort Dodge Animal Health (FDAH) does not use any substance to reduce pain or distress. Use of any such drugs therefore, would invalidate the scientific value of the protection endpoint determined by the test. Lack of confidence in the endpoint would render the test itself useless for judging vaccine potency.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

B4

NOV 27 2007

Explanation for Column E (revised)
Fort Dodge Animal Health
Registration # 42-R-0009

1. **Species:** Hamsters
2. **Number of animals achieving Cat. E in this study:** 110
3. **Explanation of the procedure producing pain and/or distress:**
Hamsters were inoculated with a virulent strain of B4 and allowed to develop clinical signs of infection in order to harvest and titrate B4 challenge material.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This animal study was conducted for the propagation and titration of B4 challenge material to be used in canine challenges for the qualification of a new reference vaccine. To produce virulent challenge material in the hamster, the animal must develop clinical signs of infection. The attending veterinarian was consulted for treatment of pain and/or distress. The pain and/or distress from this infectious disease could not be relieved because any antibiotics used to treat the disease could kill the organisms being propagated. Anesthetic, analgesic, antipyretic and non-steroidal anti-inflammatory therapy would mask or alter the progressive development of illness that is used to determine if the organism is virulent and has propagated to sufficient numbers in the animal before harvest.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**

Hamsters are required for propagation B4 challenge organisms to be used in dogs for B4 reference bacterin requalification studies. At this time B4 cannot be propagated in *in vitro* to produce virulent challenge material.

A master or working reference is necessary for *in vitro* potency testing for product release of B4 containing products. VS Memorandum 800.90 III.A. "A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity".

VS Memorandum 800.202 3.6.1 "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement".

VS Memorandum 800.202 1.3 "Efficacy is the direct effect of a medical intervention on an individual subject".

VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo-controlled, randomized, and double blinded. Vaccine trials should preferably aim to compare product- and placebo-treated subjects by their response to challenge with virulent pathogen.

JAN 14 2008

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Hamsters
2. **Number of animals achieving Cat. E in this study:** 126
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Ten hamsters are vaccinated B4 of test vaccine. Thirty hamsters are held for use as controls during the challenge. After 21 days, all vaccinated hamsters are challenged B4 of a proper dilution of B4 virus challenge material. Ten non-vaccinated hamsters are challenged B4 of the same dilution and used as challenge controls. Four groups of five non-vaccinated hamsters are given B4 of diluted challenge (to be used as a challenge titration determination). All hamsters are observed for 7 days and deaths are recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
This test is required to establish potency on each serial of vaccine produced. Death as an endpoint is the current standard and a necessary part of a valid test as determined by USDA approved Outline of Production B4. Because the vaccine is given at a fractional dose, the test amounts to a protective endpoint determination for the vaccine being tested. Furthermore, pathology and signs, length and severity of clinical disease would likely be impacted by use of non-steroidal anti-inflammatories, corticosteroids and analgesics. Use of any such drugs therefore, would invalidate the scientific value of the protection endpoint determined by the test. Lack of confidence in the endpoint would render the test itself useless for judging vaccine potency without a validated protective dose and challenge dose being determined. Until such time as a validated USDA-CVB approved alternative is available, the test is obligatory. No alternatives exist at this time, and no CVB-approved means of relieving pain and distress for this use of hamsters are yet available. When the alternatives are available to a commercially applicable scale, FDAH will apply them. FDAH has incorporated the guidelines of USDA-CVB notice No. 04-09 into the outlines of production as outlined in 9 CFR 117.4 (e)
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS 9 CFR 113.9 New potency test (a).

NOV 27 2007

**Explanation for Column E
Fort Dodge Animal Health
Registration # 42-R-0009**

1. **Species:** Hamsters
2. **Number of animals achieving Cat. E in this study:** 6966
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Ten hamsters per serial are vaccinated B4 After 14-21 days (product dependent), the hamsters are challenged B4 with an appropriate dilution of live B4 preparation. Ten non-vaccinated hamsters are given the same challenge dose and used as controls. Four groups of five non-vaccinated hamsters are given a dilution of the challenge material and used as the challenge titration determination. Hamsters are observed for 14 days, deaths recorded.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
The test is required by regulation as a proof of B4 vaccine potency to be conducted on each serial of vaccine produced. Death of hamsters in this test is used to indicate lack of protection from B4 . Because the vaccine is given at a fractional dose, the test amounts to a protective endpoint determination for the vaccine being tested. b4 in hamsters almost always results in acute onset and rapid death. The rapid progression of the disease in the hamster gives little opportunity for intervention. Furthermore, pathology and signs, length and severity of clinical disease would be impacted by use of non-steroidal anti-inflammatories, antibiotics, corticosteroids, and analgesics. For this reason Fort Dodge Animal Health (FDAH) does not use any substance to reduce pain or distress. Use of any such drugs therefore, would invalidate the scientific value of the protection endpoint determined by the test. Lack of confidence in the endpoint would render the test itself useless for judging vaccine potency.
APHIS-USDA-CVB is engaged in developing in-vitro potency test alternatives for products that require this test and FDAH has been one of the most active industry partners in this effort. Fort Dodge Animal Health has ongoing animal studies that are currently attempting to validate *in-vivo* methods for the potency tests for releasing some serials and serovars of B4 vaccines. As soon as this methodology is validated to the satisfaction of the USDA-CVB, they will be adapted into the Outlines of Production for the appropriate products. FDAH has incorporated the guidelines of USDA-CVB notice No. 04-09 into the outlines of production as outlined in 9 CFR 117.4 (e)
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
B4
APHIS 9 CFR 113.9 New potency test (a).

NOV 27 2007

Explanation for Column E (revised)
Fort Dodge Animal Health
Registration # 42-R-0009

1. **Species:** Hamsters
2. **Number of animals achieving Cat. E in this study:** 16
3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**
Hamsters in this study were anesthetized and challenged by different routes with B4 and observed for signs of clinical disease. The animals exhibited weight loss as an unexpected sign of illness. Since weight loss is a sign of possible distress, the animals were reported in column E.
4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5)**
Hamsters are an alternate animal model for B4 in dogs. Hamsters are used to determine challenge routes and doses that could be used to develop the canine challenge model for B4. The clinical sign of weight loss in these hamsters was unexpected based on previous attempts at inducing the clinical disease in hamsters. When weight loss was observed in these hamsters, it was determined they would be euthanized the next day to collect tissues to try to isolate the causative organism. No treatment for the distress of weight loss was given the day the weight loss was observed. Antibiotics would have interfered with the ability to isolate infectious organisms to determine the cause of the weight loss. In the event the weight loss was due to B4 organisms needed to be quantified in organs harvested immediately after euthanasia. Analgesics and anesthetics were not considered appropriate treatment for weight loss and their administration to an animal whose organ status was unknown could have caused premature death. Organs from hamsters dying before euthanasia could not have been used in the study.
5. **Cite the agency, code of Federal Regulations (CFR) title number and the specific section number and/or VS Memoranda that require this procedure and study.**
APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3- General Licensing Considerations: Efficacy. "Efficacy is the direct effect of a medical intervention on an individual subject."

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. "Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen."

APHIS VS Memorandum 800.202 4.2- Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.